

Women May Need More Folate

More evidence that the current Recommended Dietary Allowance of 180 micrograms a day of the B vitamin folate (folic acid) for women is probably too low has resulted from a new study by ARS researchers and their colleagues.

Adequate folate intake has been linked to decreased risk of spina bifida and other birth defects, as well as reduced risk of cardiovascular disease and stroke.

“A very small amount of the powdered form of folate,” says ARS chemist Robert A. Jacob, “provides the RDA. It’s the quantity of this nutrient found in 1-3/4 cups of orange juice or about 4/5 cup cooked red beans, for example.” Other foods rich in folate include dark-green leafy vegetables like spinach and broccoli, peas and beans, nuts and seeds, eggs, and liver.

Jacob led the folate investigation of 10 healthy, post-menopausal volunteers, age 49 to 63. They lived round-the-clock at the ARS Western Human Nutrition Research Center in San Francisco. For 9 weeks, they ate meals that provided 30 to 60 percent of the RDA for folate. For a final 3 weeks, they got meals fortified with extra folate.

The center’s dietary staff meticulously prepared and measured the volunteers’ meals. “We tried to provide familiar foods and to mimic the eating patterns of women who don’t get enough folate-containing fruits and vegetables,” says Jacob.

To remove about half of the folate from green beans, carrots, chicken, turkey, and ham, the dietary staff boiled these foods three times, discarding the water each time. Volunteers ate a standard menu of low-folate foods throughout the study, supplementing it at breakfast and dinner during the last 7 weeks

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Chemist Robert Jacob discusses progress of a 12-week folate study with participants Carol Garnett (right) and Maria Salazar.

with pure, synthetic folic acid mixed with applesauce.

A typical day’s menu might include applesauce and hash-browned potatoes or applesauce and toast with jelly for breakfast; boiled chicken, noodles, zucchini squash, and stewed tomatoes for lunch; and pasta, roast turkey, carrots with Italian dressing, and applesauce for dinner. That day’s evening snack would be a light dessert made of carrots, margarine, sugar, and a nondairy topping.

Women of childbearing age were excluded from the study because of the link between low folate levels and increased risk of spina bifida and other neural tube birth defects, that is, deformities of the brain or spinal cord. To help prevent these birth defects, the U.S. Food and Drug Administration requires—as of January 1, 1998—that enriched bread, flour, cornmeal, rice, pasta, and other grain products be fortified with folate. [See “Foods To Be Fortified With Folic Acid,” *Agricultural Research*, June 1997, pp. 16-17.]



Another Measure of Folate Status

After the 9-week regimen of low folate, the researchers found that all of the women had low levels of folate in their blood (plasma). And seven of the women had a larger number of components called micronuclei in their white blood cells.

Says Jacob, "An increase in micronuclei indicates damage to DNA in chromosomes. DNA is the genetic blueprint for all cell growth. Damage and repair to DNA is

always occurring, but chronic DNA damage may overwhelm repair mechanisms and increase the risk of cancer and birth defects.

"The micronucleus test," notes Jacob, "wasn't used to study folate nutrition in 1989, when folate RDAs for adults were lowered from 400 micrograms to 200 micrograms for men and 180 micrograms for women."

The idea of raising the folate RDA isn't new. But the San Francisco test was apparently the first to use white blood cell micronuclei counts in a controlled study of moderate folate deficiency.

The scientists used a second technique to check the body's DNA. Explains Jacob, "The body uses folate to supply one-carbon-units in many reactions, a process called methylation. That happens during formation of DNA, for instance. Methylation of white blood cell DNA decreased during the low-folate regimen. Other researchers have seen that happen in animal tests. But our experiment was the first to show that even a mild folate deficiency can reduce methylation of DNA in humans."

Seven of the 10 volunteers had elevated levels of an amino acid, homocysteine, in their blood by the time they completed the first 9 weeks of the 3-month study.

"The body," Jacob says, "has a folate-based mechanism to keep homocysteine from getting too high. It uses a form of folate called methyl-tetrahydrofolate to convert homocysteine into a different amino acid, methionine. It's another example of methylation. High homocysteine levels increase risk of heart attack or stroke."

For 2 weeks following the low-folate stint, volunteers received 160 percent of the RDA. That raised

plasma folate, but homocysteine levels remained high in half of the women.

Says Jacob, "This indicated that the current RDA may not be sufficient to maintain safe homocysteine levels in women who consume low-folate diets similar to what was fed in this experiment."

During the last week of the study, when the women were given nearly three times the RDA, homocys-

teine levels decreased to normal, or near normal, in all but one of the volunteers, whose level still remained above normal at the end of the study. Plasma folate, white blood cell micronuclei counts, and DNA methylation also returned to their pre-study levels.

Jacob did the work with ARS colleagues Denise Gretz and Peter C. Taylor at the nutrition center; Marian E. Swendseid of the University of California at Los Angeles; S. Jill

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Technician Janet Chan adds a precise amount of B vitamin folate solution to a dish of applesauce.

James of the U.S. Food and Drug Administration, Jefferson, Arkansas; Tsunenobu Tamura of the University of Alabama at Birmingham; and Nina Titenko-Holland of the University of California at Berkeley.

Folate deficiency has been linked to increased risk of cancer of the cervix, intestine, and other parts of the body that have a lining of epithelial cells. So the team also examined micronuclei counts in epithelial cells from the inside of the cheeks. Using a toothbrush, cells were gently scraped from the cheeks of each volunteer. Cheek-cell tests are used in other nutrition and toxicology studies.

Titenko-Holland found that cheek-cell micronuclei didn't increase during the low-folate phase. But they decreased after the final 3 weeks of higher folate menus.

In an earlier experiment, Jacob and Swendseid had shown that the RDA for adult males may not be adequate. Four of the 10 volunteers who ate 12 percent of the folate RDA showed moderately elevated homocysteine levels. These levels remained elevated even when the volunteers received 84 percent of the RDA.

"Raising the RDA for men—as well as women," says Jacob, "would increase the margin of safety for maintaining healthy homocysteine levels. When the folate RDA was set 8 years ago, homocysteine levels weren't measured. Nor was the connection of high homocysteine with heart disease known at that time."—By **Marcia Wood, ARS.**

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A typical meal served in the folate study included pasta, tomato sauce, and triple-boiled green beans and chicken.